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REVIEW **Open Access**

Assessment of estrogen receptor low positive status in breast cancer: Implications for pathologists and oncologists

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Summary. Estrogen receptor (ER) status assessment by immunohistochemistry (IHC) is the gold standard test for the identification of patients with breast cancer who may benefit from endocrine therapy (ET). Whilst most ER+ breast cancers have a high IHC score, about 3% of cases display a low positivity, with 1% to 10% of cells being weakly stained. These tumors are generally classified within the luminal-like category; however, their risk profile seems to be more similar to that of ER-negative breast cancers. The decision on ET for patients with a diagnosis of ER-low breast cancer should be carefully considered in light of the risks and possible benefits of the treatment. Potential pitfalls hinder pathologists and oncologists from establishing an appropriate threshold for "low positivity". Furthermore, several pre-analytical and analytical variables might trouble the pathological identification of these clinically challenging cases. In this review, we sought to discuss the adversities that can be accounted for the pathological identification of ER-low breast cancers in real-world clinical practice, and to provide practical suggestions for the perfect ER testing in light of the most updated recommendations and guidelines.

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Introduction

Estrogen receptor (ER)-mediated signaling is profoundly involved in breast cancer tumorigenesis, tumor progression, and therapy resistance (Nicolini et al., 2018; Sajjadi et al., 2021). The expression of ER is routinely tested to identify breast cancer patients who may benefit from endocrine therapy (ET) (Lopez et al., 2019; Grizzi et al., 2020). Since the beginning of the precision medicine era in breast cancer, immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded (FFPE) tissue sections has been the gold standard method for ER status assessment (Allison et al., 2020). The appropriate threshold for “positive” ER expression by IHC, however, has been traditionally a matter of controversy. During the 2021 St. Gallen

Abbreviations. ER, estrogen receptor; ET, endocrine therapy; IHC, immunohistochemistry; FFPE, formalin-fixed, paraffin-embedded; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; QC, quality controls; PgR, progesterone receptor; TILs, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancers; DIA, digital image analysis; QA, quality assurance; SOPs, standard operating procedures; IVD, in vitro diagnostic; ROI, regions of interest.

International Breast Cancer Conference, no agreement was reached on this subject, with the panel splitting fifty-fifty between 1% and 10% cutoff values (Curigliano, 2021).

The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have recently issued recommendations for reporting the results of ER IHC assays (Allison et al., 2020). These guidelines advise classifying all cases with at least 1% positive cells as ER+ (Bouchard-Fortier et al., 2017; Dixon et al., 2019; Allison et al., 2020). Whilst most ER+ breast cancers show a high IHC score, approximately 3% of cases present low ER expression, showing 1-10% weakly positive cells (Dixon et al., 2019; Harbeck et al., 2019). These tumors are now recognized as a new special category, referred to as ER-low breast cancer (Fig. 1). From the clinicopathological standpoint, ER-low invasive breast cancers are usually larger than the archetypal ER+ carcinomas (i.e. ER-high), show a higher histological grade, and, not uncommonly, basal-like gene expression profiles, with a propensity towards ET resistance (Poon et al., 2020; Sarma et al., 2020). For these patients, the decision on ET should be carefully examined based on risks and potential benefits (Allison et al., 2020). Of note, a "low positive" ER status is associated with a better response but also worse long-term outcome after neoadjuvant therapy (Prabhu et al., 2014; Yi et al., 2014; Dixon et al., 2019; Allison et al., 2020).

The optimization of the diagnostic workflow, including strict procedures in both the pre-analytical and analytical phases, quality controls (QC), focused training programs, and harmonization studies, is necessary to minimize the number of both false-negative and false-positive ER-low diagnoses (Fitzgibbons et al., 2010; Torlakovic et al., 2017). Here, we sought to provide pragmatic suggestions for the precise pathological identification of ER-low breast cancers. Particular emphasis has been given to the most updated testing methods, recommendations, and guidelines for these diagnostically and clinically challenging tumors.

Clinicopathological context

The new "ER-low" category is highly heterogeneous in terms of both clinicopathological and prognostic characteristics. Women with ER-low early breast cancer are usually younger and present with more advanced disease compared to those showing high levels of ER expression (Zhang et al., 2014). On the other hand, if compared to ER-negative diseases, ER-low breast cancers mostly affect older patients and present at earlier stages (Poon et al., 2020). At the histological examination, these tumors usually display a higher grade than those with high ER expression and are more likely to be of no-special-type histology and progesterone receptor (PgR)-negative phenotype (Yi et al., 2014; Zhang et al., 2014; Poon et al., 2020). An increased presence of tumor-infiltrating lymphocytes (TILs) and a high Ki67 labeling index have also been described (Mao et al., 2016). Patients with a diagnosis of ER-low breast cancer harbor hybrid features and intermediate risks of death and recurrence between ER-high and ER-negative types (Iwamoto et al., 2012; Nicolini et al., 2018; Benefield et al., 2020). It should be noted, however, that some groups reported almost overlapping clinical behaviors to ER-negative tumors (Raghav et al., 2012; Balduzzi et al., 2014; Yi et al., 2014). Nevertheless, a trend toward features of high aggressiveness and poor outcome was shown in all series and confirmed by the meta-analysis of Chen et al. (2018).

The clinicopathological complexity of ER-low breast cancers is reflected by their molecular heterogeneity. Genomic profiling by PAM50 showed a varied distribution of intrinsic subtypes and reported different frequencies across published studies (Iwamoto et al., 2012; Engstrøm et al., 2013; Cheang et al., 2015; Sheffield et al. 2016; Benefield et al., 2020). In particular, the basal-like subtype was the most frequently observed in the ER-low group (10.8-61.5%), followed

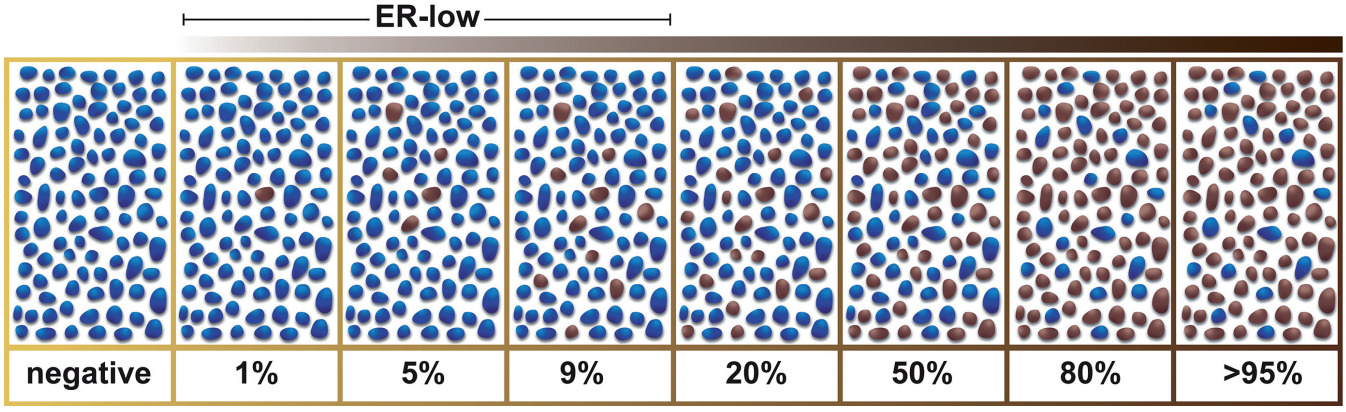

Fig. 1. ER expression spectrum with emphasis on the ER-low category. At the immunohistochemical analysis, a subset of invasive breast cancers are low ER expressors, showing 1-9% of weakly positive neoplastic cells.

by HER2-enriched (14.3-49.2%) and luminal subtypes (8.0-32.3%). High frequency of *TP53* but not phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations have also been observed (Benefield et al., 2020; Fusco et al., 2021). The median expression of estrogen receptor 1 (*ESR1*) at the mRNA level was instead lower in ER-low tumors, as well as ER-associated signatures (Iwamoto et al., 2012; Yi et al., 2014). These findings are in line with the previously reported clinicopathological features and confirm the ambiguous behavior of ER-low breast cancers. Finally, a high incidence of *BRCA* mutations has been observed in patients with an ER-low disease, similar to that of triple-negative breast cancers (TNBC) (Sanford et al., 2015; Pagni et al., 2019). In the absence of additional risk factors, if not properly identified, these patients may not undergo adequate genetic counseling and *BRCA* mutation testing.

Strategies for the precise identification of er-low status

Pre-analytical laboratory procedures

Owing to several harmonization studies and platforms' technological advances, including digital image analysis (DIA), deep learning, and artificial intelligence algorithms, both the quality and reproducibility of ER testing have improved over time (Viale et al., 2007; Engelberg et al., 2015; Torlakovic et al., 2017; Lopez et al., 2019; Invernizzi et al., 2020). In 2010, the ASCO/CAP committee reviewed the IHC antibody clones previously established for ER analysis in breast cancer (Troxell et al., 2017). These include 1D5 (mouse monoclonal), 6F11 (mouse monoclonal), ER.2.123+1D5 (mouse monoclonal antibody cocktail), SP1 (rabbit monoclonal), and EP1 (rabbit monoclonal). The 2020 ASCO/CAP update anticipated changes regarding the principles of analytic validation of IHC assays and deferred this topic to the forthcoming CAP guideline update (Allison et al., 2020). Among the available antibody clones, SP1 and EP1 have been presented with higher sensitivity and staining intensity, respectively (Diorio et al., 2016; Hicks et al., 2017; Troxell et al., 2017). The reliability of the 6F11 assay has been questioned due to the darker overall counterstain (Kornaga et al., 2016). The inter-platform and inter-clone heterogeneity in terms of staining intensity may pose additional diagnostic challenges (Diorio et al., 2016; Kornaga et al., 2016; Sinn et al., 2017; Troxell et al., 2017; Wu et al., 2018; Caruana et al., 2020).

The surgical (or bioptic) samples should be transferred to the pathology laboratory applying temperature-controlled preservation, either vacuum- or formalin-based (Berrino et al., 2020; Angerilli et al., 2021). To ensure an optimal activity and antigenicity of ER molecules, the cold ischemic time (i.e. time from the post-surgical tissue cooling for storage/transportation to the start of formalin fixation of the specimen) should not exceed 1 hour (Bussolati et al., 2015; Allison et al., 2020). At the gross examination, if the tumor is identifiable, a sample including neoplastic and normal tissue can be placed in a block and immediately fixed using 4% neutral-buffered formalin for 6-72 hours (depending on the specimen volume). The sample thickness in each block should not exceed 5 mm, while that of FFPE sections should range between 3 and 5 µm (Allison et al., 2020). Before ER testing, pathologists should select the most representative tumor sample, comprising (if available) an adequate internal control (i.e. normal breast tissue) (Torlakovic et al., 2017). External controls should include positive, negative, and low-positive (e.g. tonsil) tissue samples. Presently, a guideline update regarding IHC assay validation is under development by CAP. In the breast biomarker reporting, information on the relevant pre-analytical variables as well as the applied ER antibody clones should be provided. If internal controls are not present (but external controls are appropriately positive) an additional comment is recommended in the pathology report (Allison et al., 2020). The semiquantitative evaluation of ER nuclear expression can be reported either as the percentage of positive neoplastic cells or as discrete categories. In the case of "low-positive" ER, a review of controls, comparison of the results with any prior data, and, eventually, re-test on a different block is recommended.

During the initial validating procedures, at least 40 samples with well-known results should be tested. These samples should contain 20 negative and 20 positive cases, including at least 5 "low-positive" ER breast cancers (Nofech-Mozes et al., 2012). A concordance rate of 90% for ER+ tumors and 95% for ER-negative is considered sufficient. Documentation about validation procedures should be collected according to local regulations and importantly, any modification to each variable of the process requires additional validation (Hammond et al., 2010; Torlakovic et al., 2017). Although universal caseloads are not available, these cases should be selected by experienced breast pathologists at laboratories participating in IHC external QA accreditation programs (Hammond et al., 2010; Nofech-Mozes et al., 2012). Pathologists are encouraged to share and discuss "low-positive" ER cases. Each laboratory should be accredited for IHC by external audits and/or proficiency programs (Torlakovic et al., 2017).

Analytical challenges

The weak intensity of the ER nuclear immunostaining is a hallmark of ER-low breast cancers (Fig. 2). This phenomenon was first encountered during the enrollment phase of trials for adjuvant ET, after testing repetition by a referral center, with a frequency ranging from 10% to over 20% (Viale et al., 2007; Gelber and Gelber, 2009). Thought to be largely

imputable to the lack of national guidelines for the hormone receptors testing, most ER falsely-negative tumors were found to have pre-analytical issues, such as poor fixation (Cameron, 2009). More recently, however, some authors suggested that a subset of ER-negative breast cancers may be misdiagnosed due to artifactual low-positive staining (Caruana et al., 2020).

Good staining concordance between biopsy samples and their corresponding excision specimens has been widely documented (Burge et al., 2006; Rakha and Ellis, 2007; Usami et al., 2007; Wood et al., 2007; Hanley et al., 2009). In a large cohort of nearly 6,000 breast cancers, Nadji et al. reported that focal (i.e. heterogeneous) staining for ER was mainly related to inadequate fixation or presence of tumor necrosis (Nadji et al., 2005). This phenomenon could be recognized by a gradual loss of staining intensity from the better-fixed periphery of the tissue toward the center. By contrast, in cases with a true focal ER positivity, negative areas were usually sharply demarcated and showed more aggressive morphological features. Only a handful of studies addressing the interobserver agreement of low positive ER breast cancer have been performed so far. In a pilot multi-platform study on 264 breast cancers, a 5% discrepancy rate was reported for ER assessment (Reisenbichler et al., 2013). Not surprisingly, the majority of these discordant cases (12/13) fell in the ER-low range of positivity, while the remaining showed weak staining probably due to the ER-1D5 clone.

Over the past few years, several studies focused on the role of DIA on ER evaluation comparing its performance with the traditional ER counting (Gokhale et al., 2007; Sharangpani et al., 2007; Rexhepaj et al., 2008; Aitken et al., 2010; Lloyd et al., 2010; Tuominen et al., 2010; Nassar et al., 2011; Ali et al., 2013; Stålhammar et al. 2016; Barnes et al., 2017; Lykkegaard Andersen et al., 2018) (Table 1). Overall, DIA demonstrated similar performance compared to manual estimation. Indeed, the digital identification and the quantification (both in terms of percentage and intensity) of a nuclear marker, such as ER, is considered safe, as several DIA tools for ER assessment have obtained in vitro diagnostic (IVD) certification. These tools are particularly useful in ER-low case assessment; however, pathologists must supervise all analyses selecting the regions of interest (ROI), choosing the intensity thresholds, validating the result, and performing dedicated SOP (Nofech-Mozes et al., 2012; Allison et al., 2020). Consequently, the use of non-validated systems should be avoided. The current ASCO/CAP guidelines allow the adoption of DIA in the quantification of ER, but no clear statement is given regarding both the counting method (e.g. whole tumor slides vs hot-spots vs predetermined number of ROI) and the intensity threshold to define a nucleus as "positive". Furthermore, the studies presented in this review adopted different thresholds in the evaluation of ER and PgR positivity (≥1% positive nuclei, ≥10% positive nuclei and Allred score ≥3) and, noteworthy, no studies focused specifically on ER-low breast carcinomas. Despite these critical issues, DIA demonstrated excellent reproducibility with eyeball counting, making it a promising tool for the near future, especially considering a gradual shift towards digital pathology and large-scale whole slide scanning. Further studies are needed, especially in the setting of ER-low breast cancers.

Post-analytical workflow

The post-analytical phase is the final phase of pathology workflow in which testing results are i) evaluated before their release, ii) released timely to the

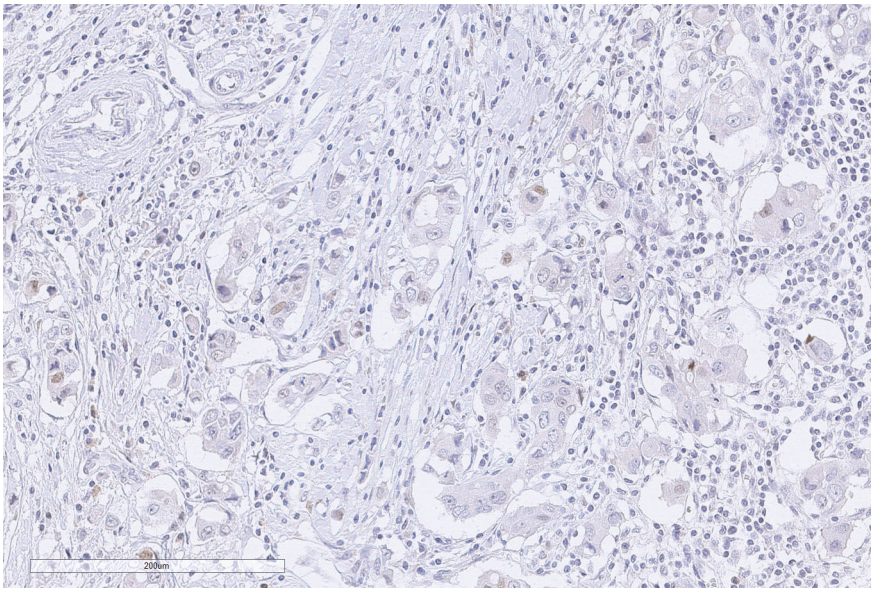

Fig. 2. Representative micrograph of a moderately differentiated invasive ductal carcinoma showing low ER expression. In this case, 5% of the neoplastic cells were positive and showed the characteristic weak staining intensity of ER-low breast cancers. Original magnification, 200x; antibody clone, SP1; platform, BenchMark Ultra, Ventana Medical Systems.

appropriate subjects, and iii) discussed to support clinical decision-making (Lenicek Krleza et al., 2019). To minimize the rate of ER-low false-positive and false-negative results, quality assurance (QA) for ER testing requires rigorous standard operating procedures (SOPs). These should describe in detail the diagnostic workflow, from the tissue excision to the ER test report (Nofech-Mozes et al., 2012; Cree et al., 2014). According to the ASCO/CAP guidelines update, ER should be tested only with a validated method and with SOPs, including scheduled pathologist competency assessment (Allison et al., 2020). Important post-analytical aspects that can be useful in case of ER re-testing are represented by sample storage and disposal, archiving of laboratory documentation, and post-analytical quality indicators. In this respect, all diagnostic phases (i.e. pre-analytical, analytical, and post-analytical phases) should be rigorously controlled, as depicted in Fig. 3.

Rationale for clinical testing

The outcomes of patients with ER-low versus ER-high breast cancers treated with ET have been evaluated in a few clinical trials (Viale et al., 2007; Francis et al., 2018). The administration of ET in patients with ER-low breast cancer was found to be beneficial only in one of the aforementioned studies. The analysis of Benefield et al. also shows that patients with ER-low breast cancer receiving ET, compared to patients with ER-high diseases, are more likely to recur, although there is no statistically significant difference. On the contrary, the outcomes of ER-low patients not receiving ET were significantly worse than ER-high and overlapping with ER-negative patients (Iwamoto et al., 2012; Yi et al., 2014; Zhang et al., 2014; Benefield et al., 2020).

Without prospective evidence, and considering conflicting data from retrospective series, administration of adjuvant ET should then be considered on a case-by-case basis, while all these patients should receive (neo)adjuvant chemotherapy. Endocrine agents are generally well-tolerated. Tolerability is highly variable and can significantly impair patients' quality of life. The balance between risks and benefits should be considered, and eventually, leading to treatment discontinuation in case of poor tolerance. If available, additional molecular assays, like ESR1 mRNA expression, may help in selecting true luminal subtypes among ER-low cases, then identifying the subgroup of patients that is most likely to gain benefit from ET.

In the metastatic setting, data about the efficacy of

Table 1. Overview of the main comparative studies between digital and manual estrogen receptor testing.

Reference	N° cases	Design	Tissue Analyzed	Scoring System	Cutoff for positivity	Statistical measurement	Results
Gokhale et al., 2007	64	DIA (ChromaVision Automated Cellular Imaging System and Applied Imaging Ariol SL-50) vs manual assessment	Surgical resections	Chromavision: Four ROIs (x40) Ariol SL-50: Four ROIs (x200) Manual: whole slide image	≥10% nuclei	Mean	No difference between DIA and manual assessment
Sharangpani et al., 2007	134	DIA vs manual assessment of ER and PgR	Surgical resections	One ROI	≥10% nuclei	Intraclass correlation coefficient	No difference between DIA and manual assessment
Rexhepaj et al., 2008	743	DIA vs manual assessment of ER and PgR	TMA	Whole TMA core	≥10% nuclei	Spearman correlation coefficient	No difference between DIA and manual assessment
Aitken et al., 2009	521	DIA Immunofluorescence) vs manual assessment of ER and PgR	TMA	Whole TMA core	Allred score ≥3	Pearson correlation coefficient	Good correlation between DIA and manual assessment
Tuominen et al., 2010	100	DIA (ImmunoRatio) vs manual assessment of ER and PgR	Surgical resections	One ROI (minimum 500 cells)	-	Pearson correlation coefficient	No difference between DIA and manual assessment
Lloyd et al., 2010	10	DIA (Definiens and Aperio) vs manual assessment of ER	Surgical resections	Whole slide image	≥1%	-	No difference between DIA and manual assessment
Nassar et al., 2011	520	DIA vs manual assessment of ER	Surgical resections	Whole slide image	≥1%	Percentage of agreement	No difference between DIA and manual assessment
Ali et al., 2013	2258	DIA vs manual assessment of ER	TMA	Whole TMA core	Allred score >2	Spearman correlation coefficient	Good correlation between DIA and manual assessment
Stålhammaret al., 2016	436	DIA (Visiopharm Integrator System) and manual assessment vs PAM50 gene assay assessment of molecular subtypes (Luminal A, Luminal B, HER2 and basal)	Surgical resections + TMA	Whole slide image (?)	≥1%	Cohen's κ correlation coefficient	DIA showed a better correlation with PAM50 gene assay compared to manual assessment
Barnes et al., 2017	354	DIA vs manual assessment of ER and PgR	Surgical resections	Minimum 3 ROIs or whole tumor section	≥1%	Overall agreement, average positive agreement, average negative agreement	No difference between DIA and manual assessment
Lykkegaard Andersen et al., 2018	112	DIA (Visiopharm VDS) vs manual assessment of ER	TMA	Whole TMA core	≥1%	Cohen's κ correlation coefficient	No difference between DIA and manual assessment

DIA, digital image analysis; ROI region of interest; ER, estrogen receptor; PgR, progesterone receptor; TMA, tissue microarray.

ET in ER-low breast cancer are virtually non-existent. Moreover, the reliability of ER assessment itself is questionable: a biopsy of the metastatic site is not always available, and when performed is prone to intratumor heterogeneity and sampling bias (Venetis et al., 2021). The combination of CDK4/6 inhibitors plus ET, the current standard first-line regimen in ER+ metastatic breast cancer, showed to retain efficacy regardless of ER levels, but only quartiles were considered (Finn et al., 2016). Regrettably, none of the randomized controlled clinical trials directly comparing the combination of targeted therapy and ET versus chemotherapy in metastatic ER+/HER2-negative breast cancers performed subgroup analysis about efficacy in

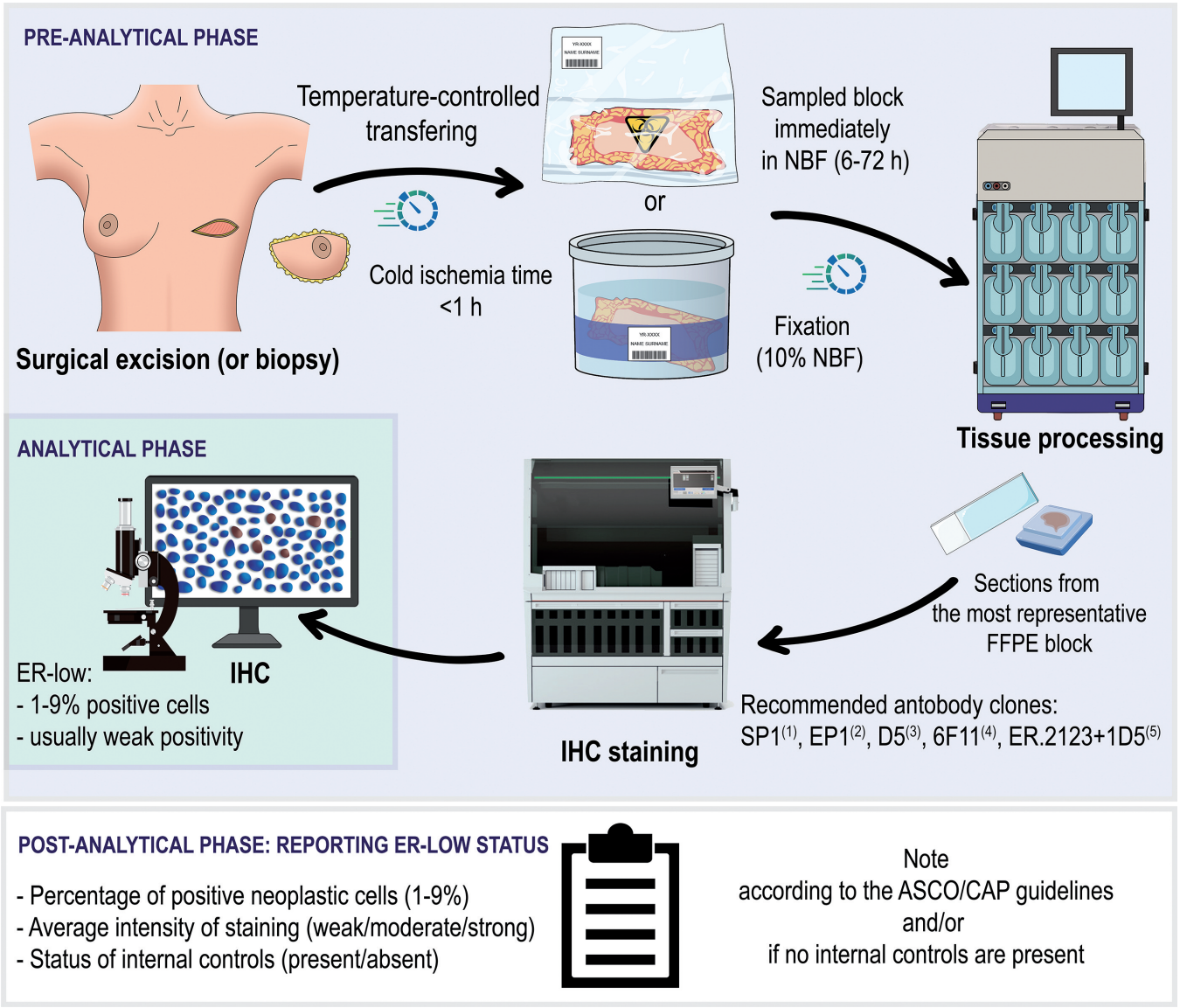

Fig. 3. Schematic representation of the standard operating procedures for an appropriate low ER status assessment. After the excision, either bioptic or surgical, the sample should be transferred to the pathology lab using a temperature-controlled system. Of note, the cold ischemia time should not exceed 1 hour. The preservation of the sample for transport can be either under vacuum or in 4% neutral buffered formalin. Time before sampling should range from 6 to 72 hours. After tissue processing, the most representative sample should be selected by the pathologist and subjected to immunohistochemistry for the analysis, which can rely on validated digital pathology tools. The biomarker report in case of low ER positivity requires information on the percentage of positive neoplastic cells, staining intensity, and status of the internal controls. According to the ASCO/CAP guidelines, a note should be added for all ER-low cases. ER, estrogen receptor; NBF, neutral buffered formalin; FFPE, formalin-fixed paraffin-embedded; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; (1) rabbit monoclonal, highest sensitivity compared to other clones; (2) mouse monoclonal, highest staining intensity compared to other clones; (3) mouse monoclonal; (4) mouse monoclonal; (5) mouse monoclonal antibody cocktail.

patients with ER-low breast cancer (Jerusalem et al., 2018; Park et al., 2019; Martin et al., 2020). According to the last ESMO guidelines, patients with ER-low metastatic breast cancer should not receive ET exclusively and could instead be considered as patients with TNBC for clinical trials, while the administration of CDK4/6 inhibitors plus ET should remain an option to be considered (Cardoso et al., 2018). Nevertheless, the same guidelines recommend considering the administration of ET whenever receptors are positive in at least one biopsy, even in case of discordance between ER expression in primary and metastatic samples. Biological variables of both primary and metastatic samples, along with tumor- and patient-related clinical features, previous clinical course, and systemic disease involvement are all elements of crucial importance when defining the specific treatment strategy for each patient with ER-low metastatic breast cancer.

Conclusions and future perspectives

The identification and treatment of ER-low breast cancer are extremely challenging for both pathologists and oncologists. According to the surrogate definition of the intrinsic molecular subtypes, these tumors are classified as luminal-like breast cancers. However, the behavior and response to treatments of ER-low breast cancers seem to be more similar to those of TNBC. In

Table 2. Ongoing and recently completed clinical trials in breast cancer patients with low levels of estrogen receptor (ER) expression.

NCT Number	ASCO/CAP Guideline	Drug	Phase	Setting	Status	Patients	Breast cancer subtype	Basket trial	Primary outcome	Secondary outcome
NCT01313039	No	AZ6244	I	E	C	4	$ER^{-/LOW}$	No	Increase ER expression	Rate of ER promoter methylation
NCT00726180	No	Trastuzumab	I/II	E	T	1	$ER^{-/LOW}$ $HER2^{+}$	No	N/A	N/A
NCT02115048	No	Olaparib + Durvalumab	I/II	E	Un	25	TNBC, ER^{LOW}	No	Changes of tumor biology	pCR, AEs
NCT03594396	No	Letrozole + Afatinib	II	E	T	44	Any	No	PFS	OS, ORR, TTP, AEs
NCT03971409	No	Avelumab + Binimetinib, Utomilumab, or PF-04518600	II	A, R	Re	150	TNBC	No	ORR	ORR, CBR, PFS, OS, AEs
NCT04265872	Yes	Bortezomib, Pembro + Cisplatin	I	A	Re	20	TNBC	No	ORR	DOR
NCT03106415	No	Pembro + Binimetinib	I/II	A	Re	38	TNBC	No	MTD	Safety and Tolerability, ORR, OS
NCT02755272	No	Pembro + Carbo + Gemcitabine	II	A	Re	87	TNBC	No	ORR, AEs	ORR, PFS, OS
NCT04249167	No	Atezo + Nab-Pacl + Cryoablation	I	A	Re	5	TNBC	No	Safety and Feasibility	IRR
NCT04468061	Yes	SG + Pembro	II	A	Re	110	TNBC, PD-L1^{-}	No	PFS	OS, ORR, DOR, TTOR, TTP, CBR
NCT03901469	No	ZEN003694 + Talazoparib	II	A, R	Re	49	TNBC	No	AEs, DLT, ORR	PFS, DOR
NCT03801369	Yes	Olaparib + Durvalumab	II	A	Re	28	TNBC	No	ORR	OS, Safety and Tolerability
NCT03853707	Yes	Ipatasertib, Carbo, Pacl, Capecitabine, Atezo	I/II	A	Re	40	TNBC	No	RP2D, PFS, OS	ORR, EFS, TTF, AEs
NCT02788981	No	Nab-Pacl + Mifepristone	II	A	Re	64	TNBC, GR^{+}	No	PFS	PFS
NCT03579472	Yes	M7824 + EM	I	A	Re	20	TNBC	No	RP2D, Safety and Tolerability	BOR, ORR
NCT02834403	No	L-NMMA + CT	I/II	A	Re	48	TNBC	No	MTD, CBR	DLT, AEs
NCT02531932	No	Carbo + Everolimus	II	A	Re	72	TNBC	No	PFS	ORR, OS, CBR
NCT03941730	Yes	Estradiol	II	A, R	Re	38	TNBC	No	CBR	AEs, PFS
NCT02926690	Yes	OTS167	I	A, R	Re	70	mBC, TNBC	No	MTD	N/A
NCT03709446	No	Leflunomide	I	A	Re	54	TNBC	No	MTD, CBR	AEs, ORR, PFS
NCT03654547	No	TT-00420	I	A	Re	75	TNBC	Yes	MTD	DRDE, OBD, AEs, ORR, DCR, DOR, PFS, OS
NCT04461600	No	AL101	II	A, R	Re	67	TNBC, Notch activated	No	ORR	CBR, DOR, PFS, OS
NCT02706392	No	ROR1 CAR T-cells	I	A, R	Re	60	TNBC, $ROR1^{+}$	Yes	AEs	ORR, PFS, OS
NCT04025216	No	MUC1 CAR T-cells	I	A	Re	112	TNBC, $MUC1^{+}$	Yes	DLT, CR, PR	AEs, OS, PFS

ASCO/CAP, American society of clinical oncology/college of American pathologists; A, advanced/metastatic; R, recurrent; E, early; Re, recruiting; Un, unknown; C, completed; T, terminated; Pembro, pembrolizumab; Carbo, carboplatin; Pacl, paclitaxel; Atezo, atezolizumab; SG, sacituzumab govitecan; EM, eribulin mesylate; CT, chemotherapy; ROR1+, receptor tyrosine kinase-like orphan receptor 1 positive; MUC1+, mucin 1 cell surface-associated; TNBC, triple-negative breast cancer; PD-L1-, programmed death ligand 1 negative; GR+ ,glucocorticoid receptor-positive; mBC, metastatic breast cancer; ER-/LOW, ER-negative/Low; HER2+, human epidermal growth factor receptor 2-positive; OS, overall survival; RP2D, recommended phase II dose; TTF, time-to-treatment failure; PFS, progression-free survival; AEs, adverse events; MTD, maximum tolerated dose; IRR, immune response rate; pCR, pathologic complete response; EFS, event-fee survival; DLT, dose-limiting toxicity; ORR, objective response rate; DOR, duration of response; DCR, disease control rate; TTOR, time to objective response; TTP, time to progression; CR, complete response; PR, partial response; BOR, best overall response; DRDE, dose recommended for dose expansion; OBD, optimal biological dose; CBR, clinical benefit rate; N/A, not available. Information has been obtained from www.clinicaltrials.gov.

the past, this empiric observation frequently led clinicians to adopt a more aggressive strategy. As yet, only a few clinical data (mainly derived from retrospective analyses) are available about the actual efficacy of ET in these patients. Hence, it is unlikely that a prospective randomized trial would ever be conducted to solve this dilemma. Clinicians should be aware of and able to discuss with patients the limited data on ER-low positive cases and the interpretability of test results that are close to a positive threshold, as stated by the recently updated ASCO/CAP guidelines. Each patient should be discussed in a multidisciplinary setting, considering both biological and clinical variables, given that ET is unlikely to be the most suitable option for all patients with ER-low breast cancer. On the other hand, without supportive clinical data, a clear threshold to withhold ET cannot be identified. In the metastatic setting, ET alone is unlikely to be the best choice, and patients should instead receive chemotherapy or combinations of ET and targeted agents. Oppositely, endocrine agents should remain at least an option to be considered for patients with early breast cancer after (neo)adjuvant chemotherapy. Also, the validation of novel assays and/or methodologies that are more efficient compared to the traditional IHC testing remains a subject of controversy (Regan et al., 2006; Allison et al., 2020). In this regard, digital pathology, deep learning, and imaging analysis algorithms represent a great innovation for surgical pathology. Prospective clinical trials specifically designed at testing the efficacy of ET in ER-low breast cancers are currently lacking (Table 2), mainly due to the small number of participants (NCT01313039, NCT00726180, NCT02115048). Additional phase I/II clinical trials on agents used as monotherapy in TNBC patients, including those with ER-low status, are in progress (NCT03941730, NCT02926690, NCT03709446). On the other hand, many studies on TNBC employed ER and/or PgR ≤10% of tumor nuclei immunoreactivity and HER2 negativity as inclusion criteria (Criscitiello et al., 2021). To the best of our knowledge, subgroup analyses have not been performed and the promotion of these trials to the next phases and publication of their results are awaited with eagerness. A common agreement among pathologists to comprehensively evaluate, report, and classify ER-low breast cancer is warranted to guide clinicians towards the implementation of the most appropriate therapy.

Conflicts of interest. N.F. has received honoraria for consulting, advisory role, and/or speaker bureau from Merck Sharp & Dohme (MSD), Boehringer Ingelheim, and Novartis. C.C. has received honoraria for consulting, advisory role, and/or speaker bureau from Pfizer, Eli-Lilly, Roche, MSD, and Novartis. C.S. has received honoraria for consulting from Roche S.p.A. These companies had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and/or in the decision to publish the results. All other authors declare no potential conflicts of interest.

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